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ANATOMY OF BARK OF BUD UNION, TRUNK, AND ROOTS OF QUICK-DECLINE-AFFECTED SWEET ORANGE TREES ON SOUR ORANGE ROOTSTOCK[1]

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INTRODUCTION

BECAUSE OF THE ECONOMIC IMPORTANCE of orange tree quick decline, the bud-union anatomy of affected trees has been under study for a number of years. Semitechnical progress reports have been issued from time to time (Schneider, 1946, 1947), but no comprehensive technical article on the subject has been published. For this reason, a technical discussion of the anatomical changes that take place at the bud union and in the trunk and roots of orange trees under the influence of this disastrous disease is presented here.

The causal agent of quick decline is graft- and insect-transmissible and is presumably a virus (Fawcett and Wallace, 1946; Dickson, Flock, and Johnson, 1951). Only trees of sweet orange, *Citrus sinensis* (Linn.) Osbeck, on stock of sour orange, *Citrus aurantium* Linn., have declined naturally in commercial plantings in southern California, and only this combination is considered here. Apparently, the virus, or some material synthesized as a result of its presence in the scion, in some way causes sieve tubes and companion cells to become necrotic as it moves across the bud union in the translocation stream. This hypothesis is based on the observation that the injury first occurs in the 8 or 10 inches of tissue below the bud union and is most severe immediately below the union. The injury to the plant is similar to that caused by dilute eosin injection (Schumacher, 1930) and in many ways it resembles normal sieve-tube degeneration. Similar injury has been observed below the bud union of buckskin-virus-infected trees of sweet cherry, *Prunus avium*, on rootstock of Mahaleb cherry, *Prunus mahaleb* (Schneider, 1945). The anatomy of the healthy phloem of sweet orange and sour orange tree trunks is reported elsewhere (Schneider, 1952, 1954*a*).

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Owing to the absence of specific external symptoms, the California State Department of Agriculture has used the anatomical symptoms as one method for diagnosing trees suspected of having quick decline (Altstatt, McClain, and Stout, 1948). Methods used for anatomical diagnoses have been described by Schneider, Wallace, and Dimitman (1950).

REVIEW OF LITERATURE

Quick decline of orange trees first attracted attention in 1939 in the Covina-Azusa area of southern California (Fawcett, 1946). In 1945, Halma, Smoyer, and Schwalm reported that the disease affected sweet orange trees on sour orange rootstock but not on sweet orange rootstock. In the same year, Fawcett (1945) reported the disappearance of starch from the rootstock of affected trees, as well as from trees artificially girdled. In some instances, in tests with iodine in potassium iodide, no color reactions were obtained in the wood below the bud union of quick-decline-diseased trees, whereas intense color reactions were obtained in the wood immediately above the bud union. In 1946, Schneider reported that sieve-tube necrosis occurred below the bud union of affected trees and that at the onset of the disease necrosis was confined to sieve-tube elements immediately below the union and appeared first in the younger sieve tubes. This suggested that the sieve tubes were being affected by something moving from the scion to the stock in the translocation stream. That same year Fawcett and Wallace (1946) reported evidence of the virus nature of the disease and its transmission by buds from affected sweet orange tops to healthy sweet orange tops on sour orange rootstock. Later, Dickson, Flock, and Johnson (1951) demonstrated transmission of the virus by *Aphis gossypii*.

Literature on diseases similar to or identical with quick decline in other parts of the world has already been reviewed (Batchelor and Webber, 1948; Bennett and Costa, 1949) and will not be repeated here. In South Africa and Java orange trees have never been successfully established on sour orange rootstock. In 1930, a disease similar to quick decline was found in Argentina. About 1937 it appeared in Brazil, where orange trees have been grown almost entirely on sour orange rootstock; there the disease is known as *tristeza*. The developmental pathological anatomy at the bud union of tristeza-affected and of quick-decline-affected mature trees appears to be the same (Schneider, Bitancourt, and Rossetti, 1947). The bud-union anatomy of trees infected by a disease which invaded Australia also seems to be the same (McAlpin *et al.*, 1948). Veinclearing and other symptoms have recently been produced in West Indian (Mexican) limes upon inoculation with the quick-decline virus. This aspect of the disease has been described by Wallace (1951), who has compared symptoms produced in lime trees in the United States with those produced in lime trees in other countries after inoculation with similar viruses occurring in those countries.

Necrosis of sieve tubes with little or no pathological change in adjacent cells as a result of virus infections is known to occur only in potato plants affected by potato phloem necrosis (Artschwager, 1923), in peach and cherry affected by buckskin disease (Schneider, 1945), and in orange trees affected by quick decline. In potato phloem necrosis and in buckskin disease of peach

and cherry, necrotic sieve tubes contain wound gum and stain red when treated with phloroglucinol and hydrochloric acid. Tests performed with these reagents on necrotic sieve tubes of orange trees affected by quick decline gave a negative reaction.

MATERIALS AND METHODS

The trees studied were 15-year-old Valencia orange scions on sour orange rootstock, growing in an orchard located between the cities of Covina and Azusa in southern California. Some of the trees were originally Washington Navel orange, but they had later been top-worked to Valencia orange and therefore had navel orange interstock. The trees not yet showing symptoms of quick decline were vigorous looking, and their trunks and bud unions were smooth. They were growing in a shallow layer of loamy sand underlaid by sand and gravel. When the study was begun in November, 1945, some of the trees were already naturally affected by quick decline. About half of the trees had previously been inoculated by budding but were not yet showing symptoms. Periodic collections of bark samples were made from trees while they were still healthy and, later, after top symptoms had appeared. Control trees were those obviously not affected in the Covina-Azusa plots and others in the Citrus Experiment Station plots at Riverside, which was outside the quick-decline area at the time.

Since none of the external symptoms of quick decline of sweet orange trees on sour orange rootstock are specific—that is, since similar decline (yellowing and loss of leaves and/or wilting) is caused by other factors—anatomical symptoms are used for diagnosis. Examinations have been made of several thousand sections of bark from trees suspected of having quick decline. These sections were prepared by members of the California State Department of Agriculture, who also determined, by the more time-consuming transmission tests, whether the trees actually had quick decline. Some quick-decline-affected bud unions from Florida and Louisiana also were studied.

Cross sections were made of bark samples taken 1 inch and 18 inches above and below the bud union, and radial sections were made of samples taken across the union. Collecting, sectioning, and staining methods used were those previously described (Schneider, 1952), except that in preparing slides for color photomicrography tannic acid was substituted for hematoxylin (method of Cheadle *et al.*, 1953) (fig. 1, *A*, *B*), or Congo red was substituted for hematoxylin (fig. 1, *D*), or lacmoid was used alone (fig. 1, *C*).

PATHOLOGICAL ANATOMY OF BARK AT BUD UNION OF MATURE TREES

Initial Necrosis of Sour Orange Sieve Tubes. To determine where and how injury occurs at the onset of the disease, bark samples from apparently healthy trees were taken at 6-week intervals across the bud union and 1 inch and 18 inches above and below the bud union. After the initial anatomical symptom of the disease had been recognized, progress of the injury was followed.

Necrosis of the younger sieve tubes and companion cells immediately below

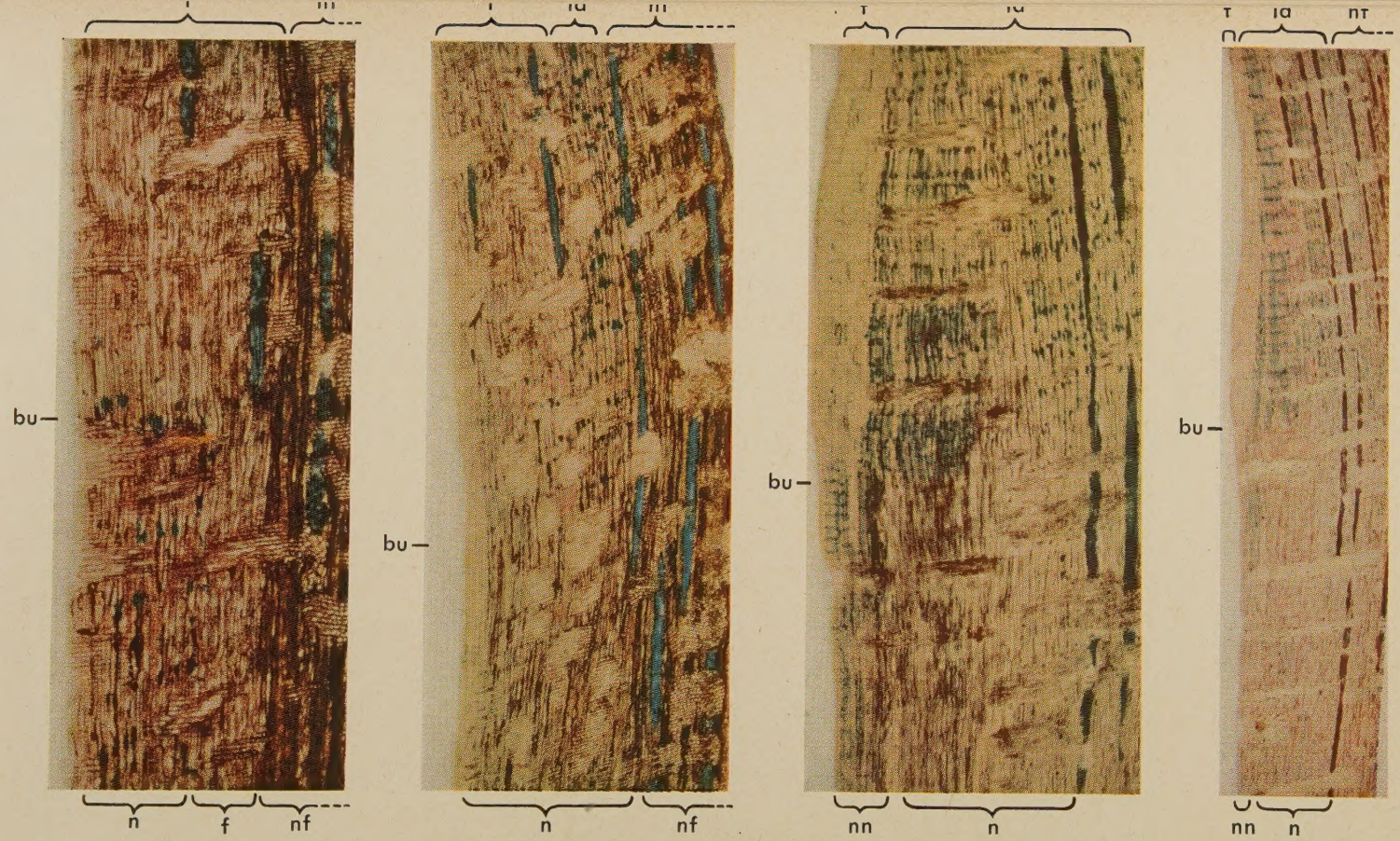


Fig. 1. Radial sections of bark samples taken across bud union (*bu*) of quick-decline-affected sweet orange trees on sour orange rootstock. (Cambium at extreme left in *A*, *B*, *C*, and *D*. Callus on sieve plates and in sieve fields stained blue.) *A*, primary stage: *above the bud union* there are normal bands of functioning (*f*) and nonfunctioning phloem (*nf*); *below the bud union* younger sieve tubes are necrotic (*n*), callus is present on sieve plates in affected areas, and parenchyma cells are hypertrophied. *B*, later stage: callus has mostly disappeared below bud union, where primary sieve-tube necrosis (*n*) occurred; sieve plates and sieve fields in older phloem above bud union are callused, and sieve-tube elements have collapsed (*id*). *C*, a stage in which new phloem has formed and callus has been deposited on new sour orange sieve tubes,

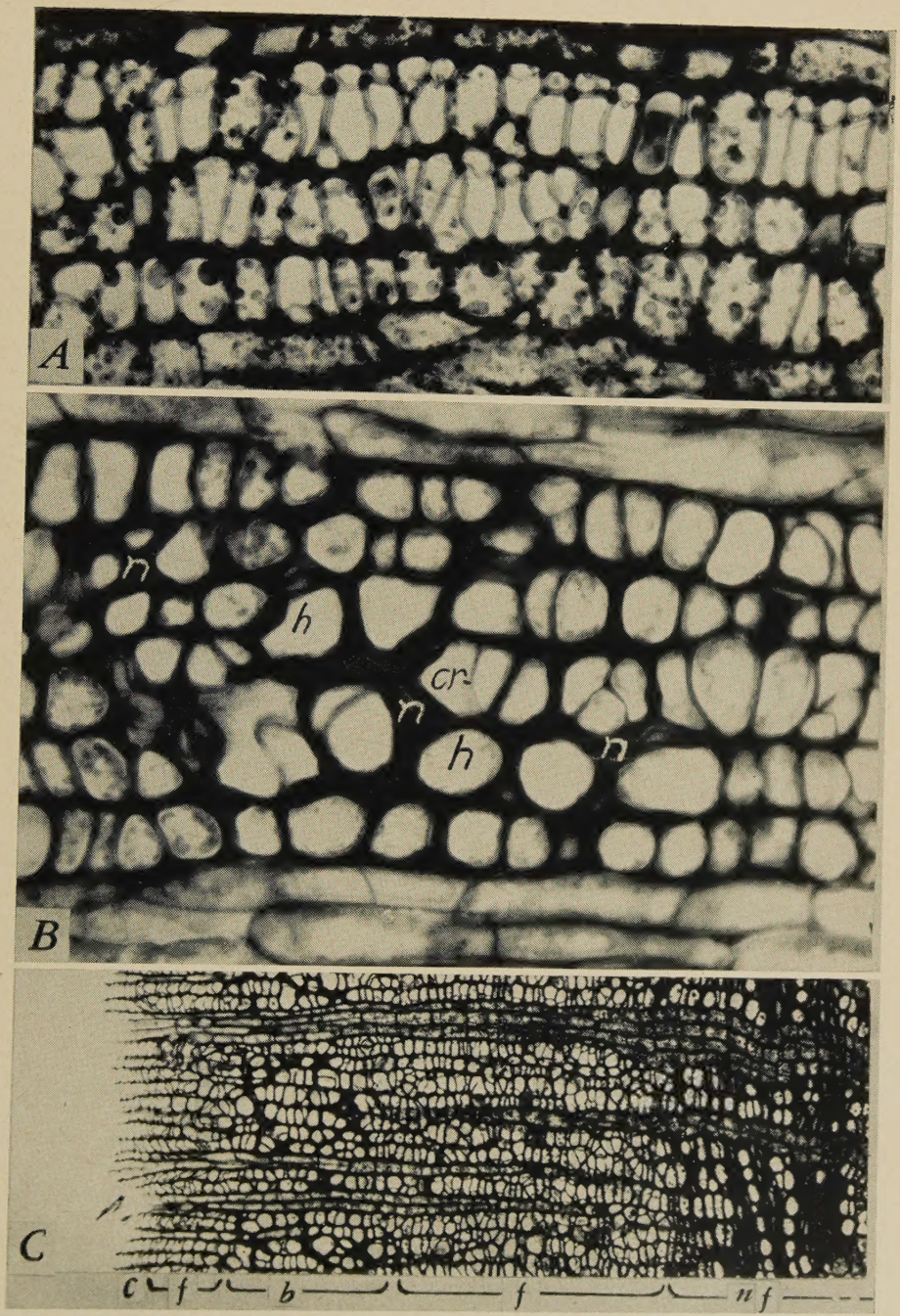


Fig. 2. Cross sections of trunk phloem of quick-decline-affected sweet orange trees on sour orange rootstock. *A*, 3/8 inch above bud union: sieve tubes (clear cells with small adjoining companion cells) normal. *B*, 3/8 inch below bud union: sieve tubes and companion cells necrotic (*n*) and crushed between hypertrophied (*h*) parenchyma cells, several of which have formed cross walls (*cr*). *C*, 1 inch below bud union: sieve tubes and companion cells necrotic in a band (*b*) through functioning phloem (*f*); normal functioning phloem (*f*) adjacent to the cambium was probably produced after necrosis occurred; nonfunctioning phloem (*nf*) is shown at extreme right; cambium (*c*) at left. (*A*, *B*, × 550; *C*, × 120.)

the bud union was the first anatomical change observed (fig. 1, *A*). The same symptom was recognized in a lesser amount 1 inch below the union (fig. 2, *B, C*) and was absent or only occasionally present 18 inches below the union.

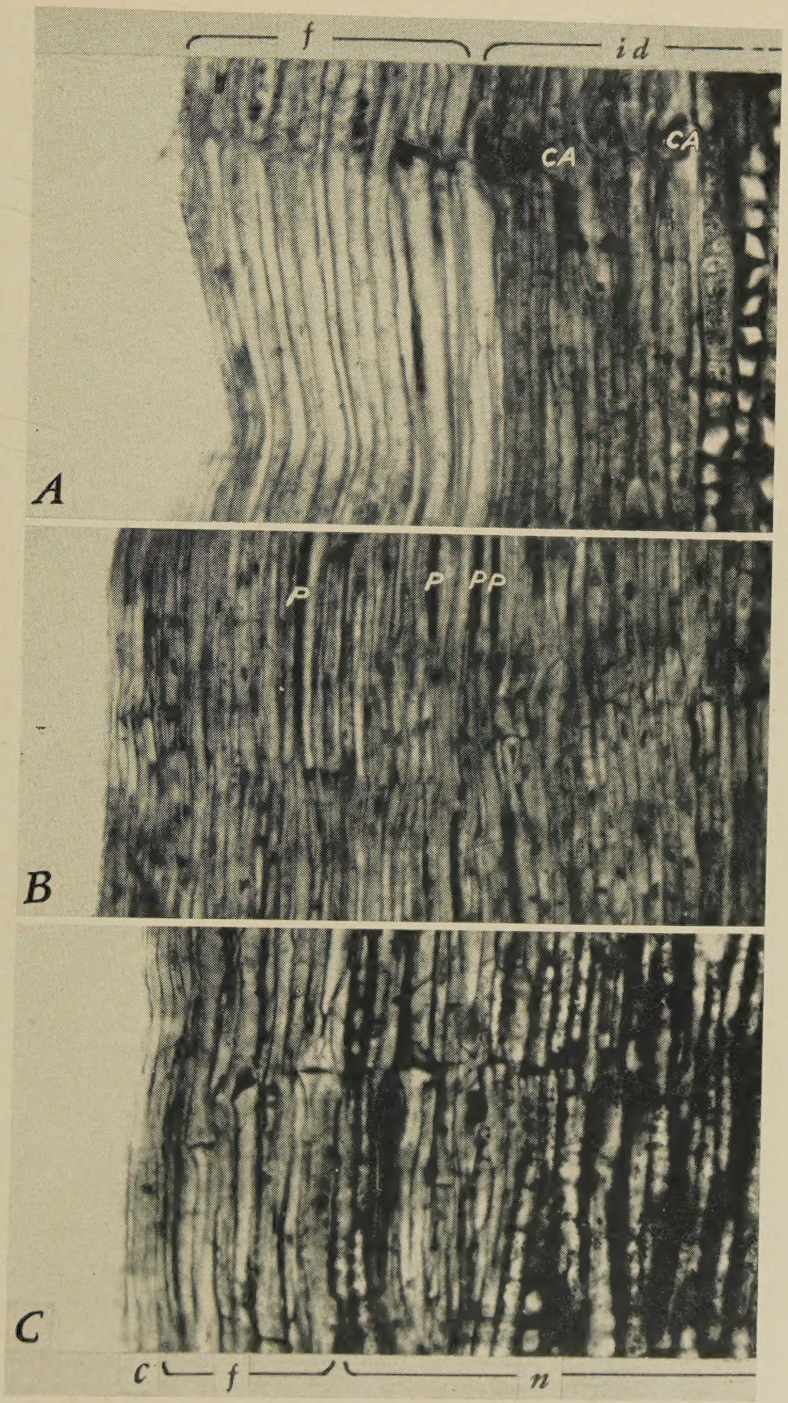
The process of necrosis of sieve tubes is similar to normal degeneration: callus appears on the sieve plates, and the sieve-tube elements lose their turgidity and collapse. Hypertrophy of adjacent parenchyma cells may accompany sieve-tube necrosis and result in crushing of necrotic sieve tubes (fig. 2, *B*). It is typical for the first and most severe injury to occur near the cambium, where hypertrophy of the parenchyma is most pronounced. Ray cells are not affected in the early stages of the disease.

Anatomical Changes Subsequent to Initial Sieve-Tube Necrosis. After the initial necrosis of the sieve tubes immediately below the bud union, reactions occur in the vicinity of the bud union. Immediately above the bud union the older sieve tubes of the scion begin to degenerate (figs. 1, *B;* 3, *A*). Callus appears on the sieve plates, and the sieve tubes lose their turgidity and collapse, with little or no hypertrophy of adjacent parenchyma cells. Sieve-tube degeneration progresses upward from the bud union. While sieve-plate callus is forming above the union, necrotic changes below the union have proceeded farther, the callus is removed from sieve plates of necrotic sieve tubes, and fat globules are gradually cleared from the parenchyma cells.

One of the most striking aftereffects of sieve-tube necrosis is the stimulation of cambial activity in the vicinity of the bud union. An excessive amount of phloem is produced, in which the cells do not attain normal size (figs. 1, *C, D;* 3, *B*). If initial necrosis occurs during the growing season, when the cambium is active, accelerated cambial activity immediately follows necrosis of the sour orange sieve tubes. If initial necrosis occurs in the winter, intensified cambial activity is delayed several months until cambial awakening in the spring. The sour orange sieve tubes in this new hyperplastic phloem sooner or later become necrotic, and the processes secondary to the necrosis described above repeat themselves in the new phloem tissues (fig. 1, *C, D*). The hyperplastic phloem tissues in the immediate vicinity of the bud union differ from normal: fibers usually do not develop therein; the ray cell initials derived from the cambium may fail to elongate radially to a normal degree; and heavily staining vacuolar material occurs in some parenchyma cells in the new phloem directly above the bud union (fig. 3, *B*).

Acropetal to the hyperplastic phloem that generally forms, there is usually a band of normal phloem (fig. 3, *A*). Below the bud union the rays occasionally become hyperplastic (fig. 4, *A*). Cells of the hyperplastic rays may develop secondary walls, and the walls may become lignified. When the bark is removed, such woody hyperplastic rays may remain attached to the xylem;

Fig. 3. Radial sections of phloem of quick-decline-affected sweet orange trees on sour orange rootstock. *A*, ¼ inch above bud union: (*left*) band of functioning phloem (*f*) with normal-sized cells; (*right*) area in which sieve tubes apparently were induced to degenerate (*id*) as a result of an initial necrosis below the union; callus (*ca*) was still present on the sieve plates. *B*, immediately above bud union: excessive amount of phloem with undersized cells, some cells filled with heavily staining material (*p*). *C*, ¼ inch below bud union: cells of normal size but only a few functioning sieve tubes (*f*) near cambium (*c*); in the area on the right (*n*), older sieve tubes and companion cells were necrotic. (All × 260.)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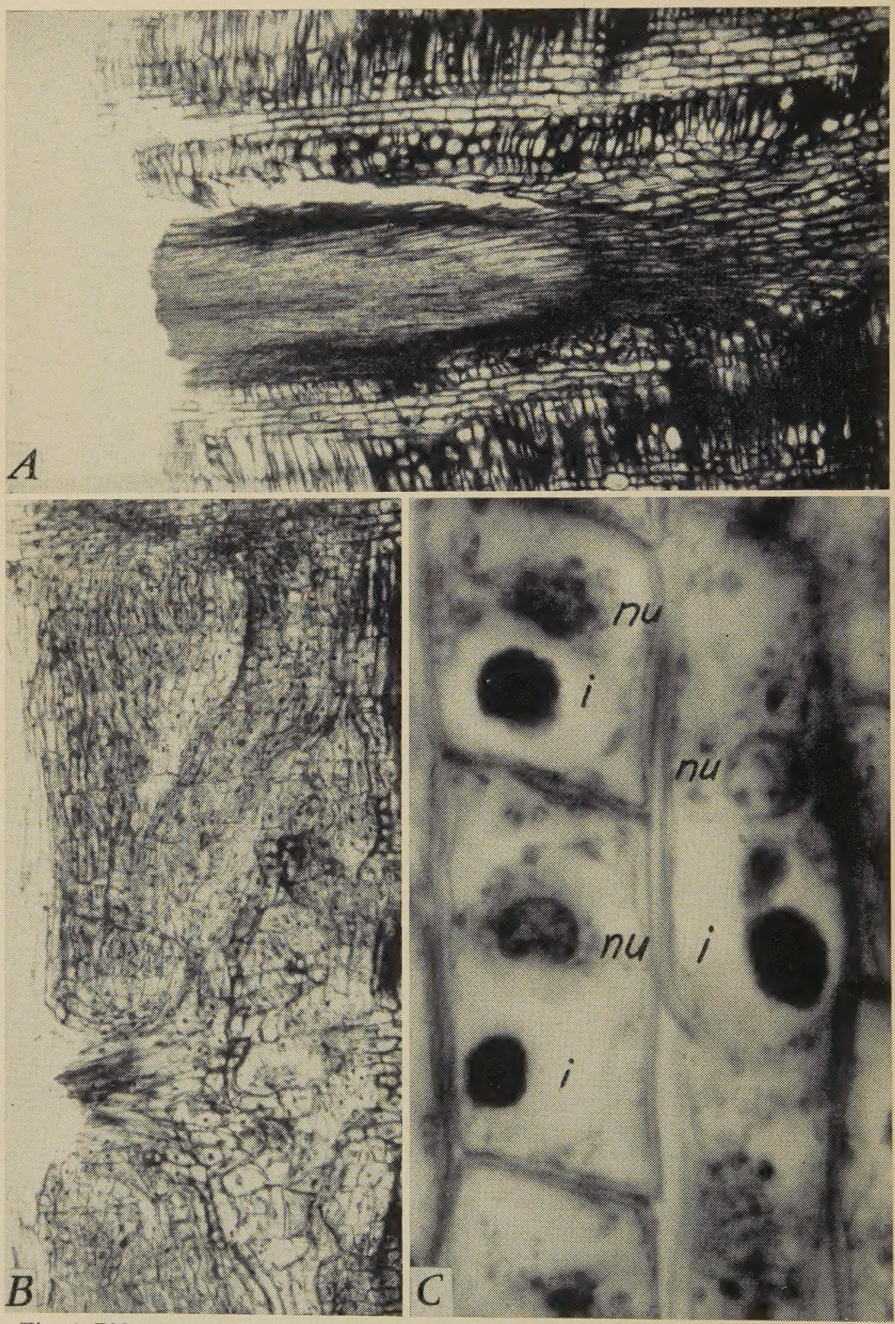

Fig. 4. Phloem of quick-decline-affected sweet orange tree on sour orange rootstock. *A*, radial section of hyperplastic lignified ray from below bud union (cambium at left). *B*, radial section of callus tissue formed above bud union by division of ray and parenchyma cells (cambium at left), *C*, inclusion bodies (*i*) and nucleus (*nu*) in parenchyma cells in vicinity of bud union. (*A*, *B*, × 120; *C*, × 1700.)

small holes therefore occur on the cambial face of the bark. Above the bud union the ray and phloem parenchyma may become hyperplastic at times to form a calluslike tissue (fig. 4, *B*). The calluslike tissue formed in quick-decline trees sometimes becomes necrotic, takes on a brown color which is visible macroscopically, and adheres to the face of the wood just above the bud union when the bark is removed.

Inclusion bodies such as occur in virus-diseased plant tissues have been observed in ray and phloem parenchyma cells in the vicinity of the bud union (fig. 4, *C*).

PHLOEM 1 INCH AND 18 INCHES ABOVE AND BELOW THE BUD UNION

Bud unions of the experimental trees were a few inches above the ground. Bark samples for sectioning were taken 1 inch above and 1 inch below the bud union, as well as 18 inches above the union (on the trunk or a large limb) and

TABLE 1

AVERAGE RADIAL WIDTH (IN MICRONS) OF FUNCTIONING PHLOEM IN BARK SAMPLES FROM TRUNKS AND ROOTS OF ORANGE TREES VARIOUSLY AFFECTED BY QUICK DECLINE

Bark samples, distance from bud union	Healthy trees (27 samples)	Tree tops normal, but sieve tubes necrotic (23 samples)	Top symptoms			
			Suspected (9 samples)	In early stage (10 samples)	Moderately advanced (19 samples)	Advanced (13 samples)
	μ	μ	μ	μ	μ	μ
18 inches above*	816	760	557	455	388	278
1 inch above	725	593	427	244	250	234
1 inch below	625	126	83	67	136	79
18 inches below†	310	264	203	126	131	79

* Samples taken from trunk or large limbs.
† Samples taken from large roots.

18 inches below the union (on roots of large diameter). The radial width of the band of functioning phloem in these areas—namely, that portion of the phloem having sieve tubes which were mature but had not degenerated or become necrotic—was measured in microns. Results are presented in table 1.

The trees from which bark samples were taken may be classified as follows: (1) healthy; (2) without top symptoms of quick decline, but sieve tubes necrotic; (3) top symptoms of quick decline suspected; (4) disease in early stage (leaves gray-colored; some wilted); (5) disease in moderately advanced stage; (6) disease in advanced stage; and (7) trees in stage of equilibrium (nearly defoliated but putting out limited new dark-green growth from large branches). These categories were set up for trees that go through progressive stages of the disease until they die or reach a stage of equilibrium. Occasionally, however, trees begin to decline and then partially recuperate. The various "stages" may therefore indicate intensity of symptoms rather than the length of time the trees have been diseased.

The most striking difference between sections of bark samples of healthy and of diseased trees was the reduction in amount of functioning phloem as a result of sieve-tube necrosis 1 inch below the bud union of diseased trees (see table 1 and fig. 5). Before the appearance of top symptoms, necrosis was usually found only for a distance of about 10 inches below the bud union, the phloem at other points of examination being affected little or not at all. At its onset, sieve-tube necrosis was frequently near the cambium (fig. 2, *C*).

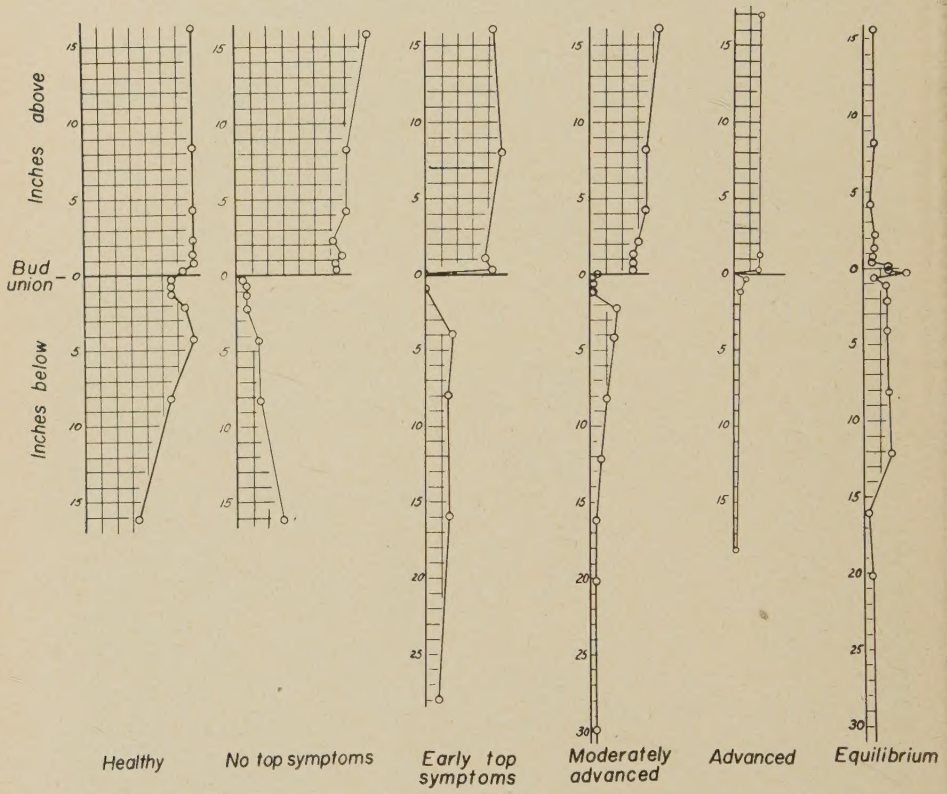

Fig. 5. Diagrams showing extent of functioning phloem (crosshatched area) in bark of a healthy sweet orange tree on sour orange rootstock, and of trees in various stages of quick decline. The vertical line at the extreme left in each diagram represents the cambium. Circles indicate locations of cross sections made for phloem measurements. Distance between vertical lines, 100μ; distance between horizontal lines, 1 inch.

Necrosis of the older sieve tubes succeeded that of the younger tubes, and there was less tendency toward hypertrophy of parenchyma cells adjacent to the older tubes. At the 18-inch level below the bud union, only occasional sieve tubes became necrotic at the onset of the disease. In roots depleted of stored starch another type of sieve-tube degeneration sometimes occurred, which affected all the sieve tubes. This type of degeneration also developed in trees girdled by ringing and not affected by quick decline (Schneider, 1954*b*).

At an average of 12 months after the appearance of the initial anatomical symptoms, when top symptoms were just beginning to appear, the most ex-

tensive necrosis was still found in the tissues 1 inch below the bud union, but the older sieve tubes 1 inch above the union were then beginning to degenerate. In the more advanced stages of the disease, the band of functioning phloem became narrower throughout the trunk but was narrowest in the vicinity of the bud union. The reduction in width 18 inches above the union was probably an indirect effect of tree deterioration.

TIME LAG BETWEEN APPEARANCE OF SIEVE-TUBE NECROSIS AND OF TOP SYMPTOMS

Since there is a considerable amount of reserve starch in the rootstock of orange trees, sieve-tube continuity between the top and the roots of a tree may be completely interrupted by experimental ringing or by other means for several months before any outward effects are shown by the tree (Fawcett, 1946; Schneider, 1954*b*). The incomplete girdling caused by quick decline allows an even longer period between the onset of sieve-tube necrosis and top symptoms.

Apparently healthy trees were selected at random, and phloem studies were made at various times of the year. Sieve tubes of the phloem of 38 of the trees were in an early stage of necrosis, although necrosis had been absent in some of the trees when they were sampled a few weeks earlier. The period between the beginning of necrosis and the appearance of definite decline symptoms in the tops, which were graded for symptoms and recorded at 3-month intervals, was as long as 23 months for one tree. Where the period was extended, premature coloring of fruit was observed long before there was evidence of tree decline. The average length of time between the beginning of necrosis and the first top symptoms was 11½ months.

Random sampling of apparently healthy trees on different sides of the trunk at the bud union showed five trees with necrosis on one side of the trunk and not on the other. Apparently, the trees became infected on one side, and it was several months before the virus invaded the entire tree.

SEASONAL VARIATION IN AMOUNTS OF FUNCTIONING AND DEVELOPING PHLOEM

A study was made of the amount of functioning phloem immediately below the bud union of diseased trees at various times of the year. Radial measurements were made of this phloem, which was newly produced, functioned for a short time, and then became necrotic. Results of these measurements are presented graphically in figure 6, *A*. Each point on the curves represents the average amount of phloem in five trees, determinations being made from one radial bud-union section per tree. The top symptoms of the trees ranged from very early stages to advanced stages of decline. The width of functioning phloem present in all the samples throughout the year ranged from 0 to 250μ. The average amount of functioning phloem for all dates for both years was 70μ. As stated above, the hyperplastic phloem contained cells of a size that was smaller than normal.

When phloem development is active, slime bodies are conspicuous in differentiating sieve tubes. These slime bodies are diffuse colloidal structures

which disintegrate to form the sieve-tube slime of mature sieve tubes, and their presence appears to be the best indication of phloem formation. Curves showing the number of slime bodies present in the phloem of diseased and healthy trees at various times of the year are presented in figure 6, *B*. The

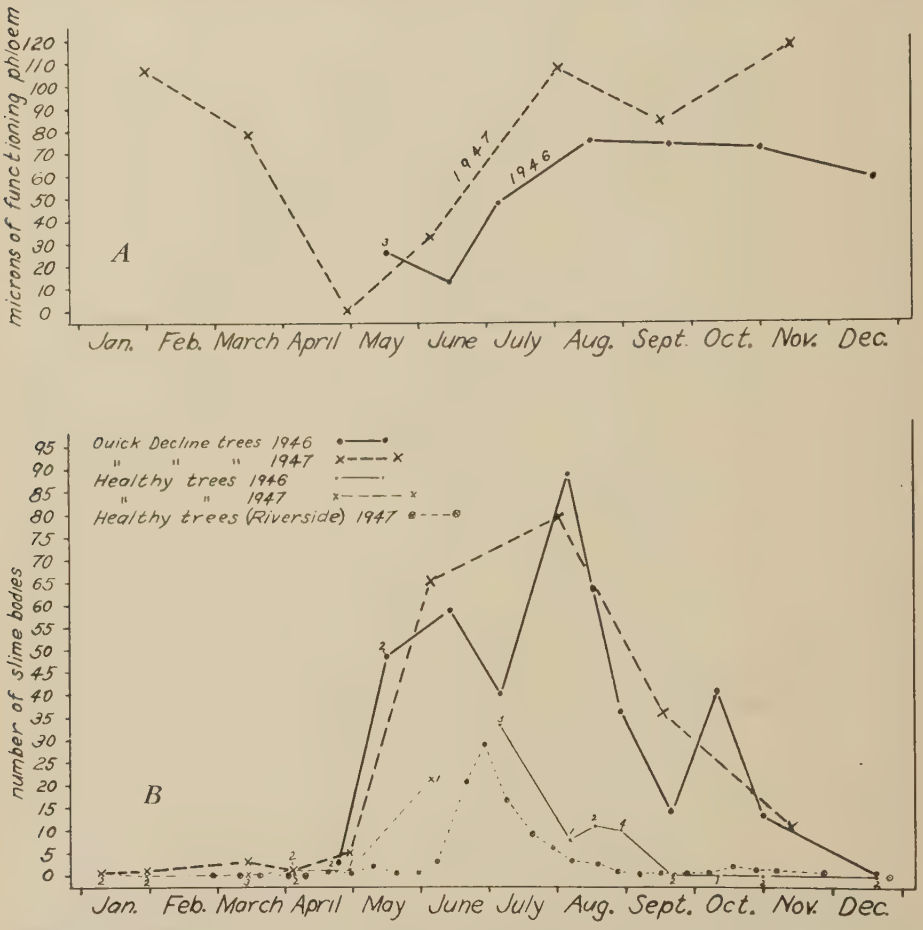

Fig. 6. *A*, average amount of functioning phloem immediately below bud union in bark samples from orange trees variously affected with quick decline, 1946 and 1947. (Each point on these curves represents average of five trees or of number of trees specified by given numeral.) *B*, total number of slime bodies per four radial sections from bark samples taken at bud unions of sweet orange trees on sour orange stock, except for healthy (control) trees at Riverside, which were sweet orange on sweet orange stock and were not sampled at bud union. (Each point on these curves represents average of six diseased trees, or of five Riverside control trees, or of number of trees specified by given numerals.)

slime bodies present in four radial sections approximately 20μ thick and 1 cm long were counted for each sample. The maximum number of slime bodies found in four sections from diseased trees was 178, as compared with 39 in sections from healthy trees. Both the intensity and the duration of sieve-tube production are greater in diseased trees than in healthy ones.

INCUBATION PERIOD FOR SIEVE-TUBE NECROSIS IN YOUNG SWEET ORANGE TREES ON SOUR ORANGE ROOTSTOCKS

In an attempt to determine how long a period is required for sieve-tube necrosis to develop after inoculation, nursery-sized healthy sweet orange trees on sour orange stock were inoculated by budding. Bark sampling was begun 5 months later, and was continued at approximately 4-week intervals. The trees used were from a transmission experiment conducted by H. S. Fawcett and J. M. Wallace. The trees were planted in the field in November, 1945; they were inoculated in July, 1946, and sampling was begun December 3, 1946. Because the trees were small, the periodic samples could not all be taken from the same trees; some of the trees were sampled on two collecting dates, however.

On December 3, 5 months after inoculation, six inoculated trees were observed to have phloem which was in as good condition as that of the check trees. On February 10, 7 months after inoculation, two trees out of ten had developed sieve-tube necrosis below the union. On March 13 definite sieve-tube necrosis was present in seven trees and there was some question about three others. Of twenty trees sampled on April 28, twelve had definite necrosis and two were questionable. Definite necrosis was present on June 21, in two of ten trees (different trees were sampled on each date), and on August 6, seven of ten had definite necrosis. This indicates that it takes about 7 months or longer for sieve-tube necrosis to appear in 2- to 3-year-old trees after bud inoculation in the scaffold branches.

Detection of early necrosis in young budded trees is occasionally difficult because there are only a few sieve tubes in the band of functioning phloem of small, recently planted trees, and if half of these sieve tubes become necrotic owing to quick decline, they are hardly noticeable. Also, new phloem formed in affected trees may not become necrotic immediately. In this respect young trees differ from mature trees. In mature trees the comparatively wide band of phloem normally present in healthy trees is only partially replaced after sieve tubes become necrotic, and then with a new phloem tissue composed of small cells (fig. 3, *B*). One young tree showing sieve-tube necrosis in February had, in June, a normal band of functioning and developing phloem (indicated by slime bodies and new fibers without secondary walls), but by August the tree was showing top symptoms of quick decline.

SUMMARY

Anatomical disturbances in sweet orange trees on sour orange rootstock infected by the quick-decline virus were investigated. The first anatomical symptom is necrosis of sieve tubes and companion cells immediately below the bud union. The youngest sieve tubes are usually affected first. Adjacent parenchyma cells hypertrophy and may divide once or twice.

Anatomic changes in the vicinity of the bud union after the initial necrosis of sieve tubes are as follows: degeneration of the older sieve tubes and companion cells above the union; an overactivity of phloem production

at the bud union, where phloem cells are smaller than normal; and, occasionally, the production of calluslike tissue above the union. In addition, the rays below the union may become hyperplastic, and the walls of such ray cells may become thickened and lignified. Inclusion bodies are sometimes present in parenchyma cells at the bud union.

One fourth of an inch above the union there is usually a band of normal, functioning phloem, which, however, is narrower than in healthy trees. Immediately below the union a much narrower band of functioning phloem composed of small cells is usually found. One fourth of an inch below the union there may be a few sieve tubes of normal size adjacent to the cambium.

Farther from the bud union (1 inch or more above), the phloem remains normal until the tree begins to deteriorate.

Eighteen inches below the bud union, in the roots, necrosis of sieve tubes and hypertrophy of parenchyma occasionally occur. As the starch becomes depleted in the roots, degeneration of sieve tubes at times becomes general. A similar condition has been found in trees artificially ringed.

The width of the functioning phloem immediately below the bud union of trees showing decline symptoms ranged from 0 to 250μ (average width, 70μ).

Some functioning sieve tubes immediately below the bud union were present in most of the trees throughout the year, except for a short period in the spring when phloem formation was beginning and necrosis was almost complete.

Phloem production at the bud union was more intense in diseased trees than in healthy trees and it continued over a longer period.

Necrosis appeared in 2- to 3-year-old orange trees about 7 months after inoculation by budding.

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CONDITION OF PHLOEM OF SOUR ORANGE TREE TRUNK IN WINTER[1]

HENRY SCHNEIDER[2]

INTRODUCTION

THE PURPOSE of the present study was to determine whether or not the sour orange tree, *Citrus aurantium* Linn., maintains throughout the winter a band of functioning phloem which is equal in width to that found in the summer. This work was undertaken in connection with a study of quick-decline-affected sweet orange trees on sour orange rootstock. Sieve tubes of the sour orange stock of such trees become necrotic for a short distance below the bud union (Schneider, 1954). Because of the similarity of necrosis and normal degeneration of sieve tubes, it was important to determine whether normal seasonal degeneration, which might be confused with sieve-tube necrosis, occurs in extensive amounts in trunks of sour orange trees and in sour orange stocks of sweet orange trees.

The various zones of the trunk phloem of the sour orange at different seasons of the year are here compared with those of the sweet orange, *Citrus sinensis* (Linn.) Osbeck, described earlier (Schneider, 1952). At some seasons of the year there is present in typical sweet orange bark a narrow zone of developing phloem (phloem that is differentiating from cambial derivatives into functioning phloem). Throughout the year there is a zone of functioning phloem about 500 to 1,000μ wide, a relatively narrow zone of degenerating phloem (phloem in which sieve tubes and parenchyma are degenerating), and a vast area of nonfunctioning phloem (phloem in which the sieve tubes and some parenchyma cells have degenerated).

In deciduous trees it is characteristic for all but possibly a few small sieve tubes near the cambium to undergo degeneration after leaf fall and then be crushed by radial growth the following year (Esau, 1950).

MATERIALS AND METHODS

Bark samples from trunks of sour orange trees were collected in test plots at the Citrus Experiment Station at Riverside. Most of the samples were taken from five 11-year-old seedling trees. Five 12-year-old sweet orange seedling trees were sampled at the same time for comparison.

Trees of sour orange rootstock under sweet orange tops were sampled in two orchards at the Citrus Experiment Station at Riverside and in one orchard near Covina. Bark samples were taken 1 inch and 18 inches above and below the bud union. Since the bud union is located a few inches above the ground, bark from 18 inches below the bud union was from roots.

The rootstock in one orchard at the Citrus Experiment Station was of the

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Paraguay variety of sour orange, which belongs in the Bittersweet group. Data and evidence available indicated that rootstocks in the other orchards belonged to the normal group of sour orange varieties. (See Webber and Batchelor, 1946, for descriptions of these groups.)

Methods of collecting samples and of fixing and staining sections were the same as those described previously (Schneider, 1952). Bark samples were fixed in Randolph's modified Navashin's solution, sectioned on a freezing microtome, stained progressively with Heidenhain's hematoxylin, counterstained with lacmoid, and mounted in Canada balsam after dehydration in an ethyl alcohol series. (For further details, see Schneider, 1952.)

OBSERVATIONS

Commercially grown citrus varieties are usually budded onto a seedling rootstock several inches above the ground level. After the bud has grown out and the stem has reached a height of several feet, the young tree is topped about 30 inches above the ground and lateral (scaffold) branches are encouraged to grow out. This process is called "heading." As the tree grows, the trunk, especially the upper part, may develop furrows. The base of the trunk is usually more rounded than the part immediately below the scaffold branches, but the occurrence of large lateral roots near the ground level may result in furrowing at the base of the trunk. Therefore, part of the bark of the trunk may be in concavities and the rest on ridges.

Enlargement of the tree trunk by divisions of the vascular cambium results in stresses of different kinds in the concave and convex portions of the bark. On the ridges or convex portions there are tangential stresses and radial compression, which cause the degenerated sieve tubes and parenchyma cells of the nonfunctioning phloem to be stretched tangentially and flattened (fig. 1). The living rays may become twisted or bent in the nonfunctioning phloem, and the ray cells may become compressed in such a way that their radial walls become accordionlike. Cells of other rays become stretched tangentially, and divide repeatedly to form blocks of living parenchyma in which groups of sclereids may form (fig. 2, *A*). In the bark on the ridges, functioning and nonfunctioning phloem stand out in bold contrast to each other because the cells of the nonfunctioning phloem are flattened almost as soon as they have degenerated.

In the furrows or concave portions of bark, on the other hand, there may be radial tensions. Under these conditions the rays remain straight in the nonfunctioning phloem; the ray cells are not stretched tangentially, nor do they divide. In the blocks of nonfunctioning phloem between the rays in these areas, there is less tendency for the parenchyma cells to degenerate, and the nonfunctioning sieve tubes are crushed less completely or not at all.

Bark samples for this and other work by this writer have, wherever possible, been taken from convex portions of the trunk. Only occasionally has it been necessary to sample flat portions, where cambial growth may not have been so active, and where radial compression may not have been so great as on convex portions. The trunks of trees sampled in this study were fairly rounded and free of fluting.

It is presumed for the purposes of the present study that the radial width

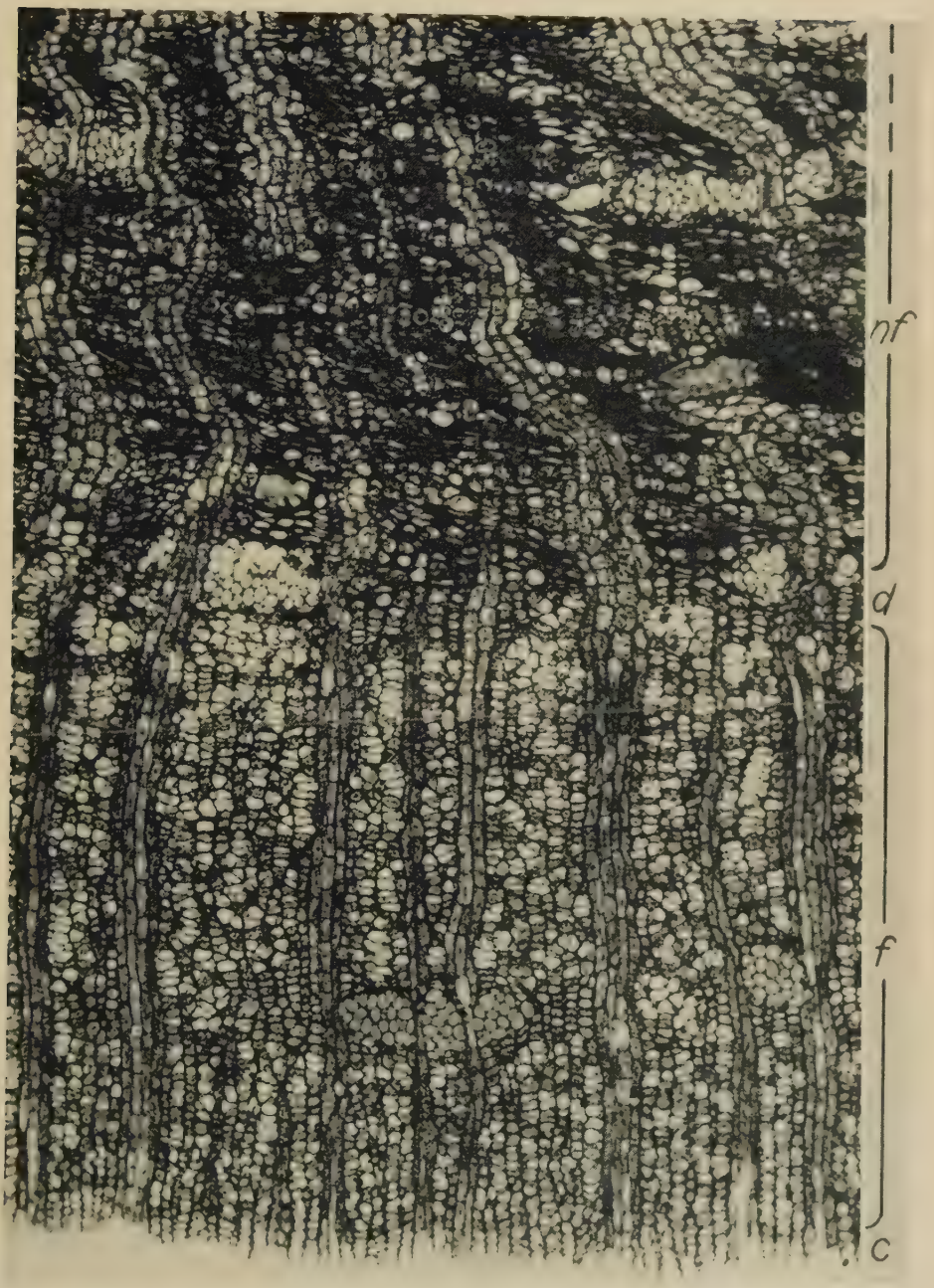
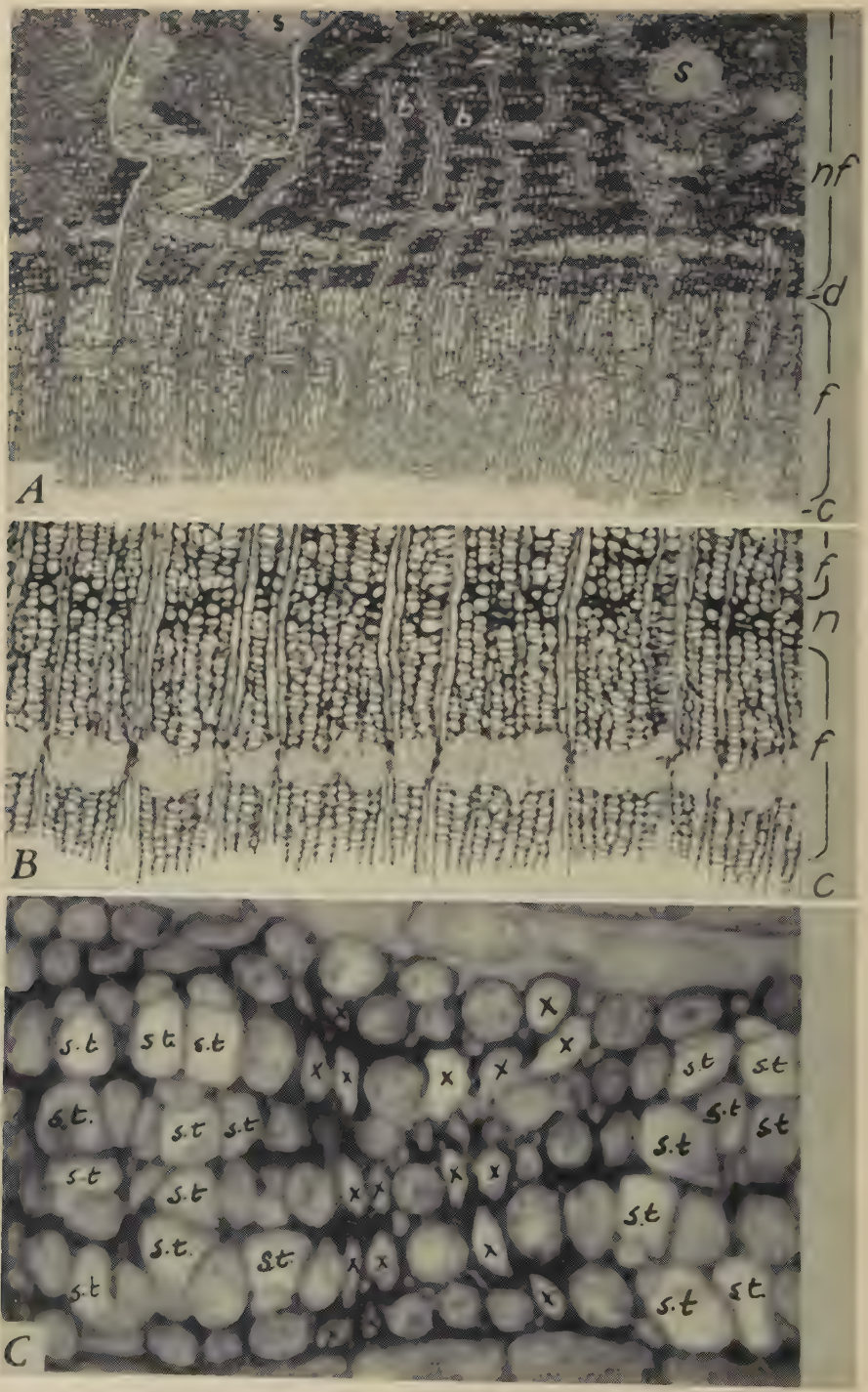


Fig. 1. Cross section of inner bark of sour orange tree trunk, showing cambium (*c*), functioning phloem (*f*), degenerating phloem (*d*), and nonfunctioning phloem (*nf*).(× 120.)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of blocks of functioning phloem between rays is uniform in any one section (fig. 2, *A*), but that there is some variation in width on different sides or in different parts of the trunk. It is also presumed that the ring of degenerating phloem should not normally be more than 50 to 100μ wide at any time. Sieve-tube degeneration supposedly occurs only on the outer margin of the functioning phloem, and degenerating sieve tubes are therefore not scattered throughout the functioning phloem.

When necrotic sieve tubes occur abnormally in the functioning phloem, they are, in their final stages of necrosis, folded masses of walls between expanded parenchyma cells. Under the low power of the microscope such dead cells appear to be masses of structureless, heavily staining material, but the crushed, folded walls may be seen under higher power. At times it is impossible to determine whether such masses of material are sieve tubes, parenchyma cells, or a combination of both. In some instances, the origin of the material may be recognized as sieve tubes by the sieve-plate callus, if it has not yet been eroded away.

Other heavily staining masses may occur in the functioning phloem. Clusters of dead parenchyma cells have been observed in the phloem of both the sweet and the sour orange. That these are parenchyma cells is deduced from the fact that clusters of cells which have died, but which have not yet been crushed, have no companion cells (fig. 2, *C*). Sieve-plate callus has not been observed on such cells. Crystal idioblasts are also heavily staining masses, but an idioblast is easily recognized by the rhomboidal calcium oxalate crystal embedded in the heavily staining matrix. Although crystal idioblasts are usually found around fibers, those dispersed throughout the phloem vary from few in most sections to many in others. Other heavily staining masses appear at times when cells of the outer functioning phloem are pulled apart by the tangential stresses set up by radial growth. The pectic middle lamella increases in amount under these conditions. Such intercellular masses of heavily staining materials may be distinguished from dead cells by the absence of folded walls.

Trunk Phloem of Sour and Sweet Orange Trees Grown from Seed. In two sour orange trees sampled at Riverside March 1, 1946, before cambial awakening from winter dormancy, bands of functioning phloem about 500μ in width were separated only by occasional degenerating sieve tubes from the tangentially flattened nonfunctioning phloem (fig. 1).

Five other sour orange seedlings were sampled on five dates between November 18, 1946, and April 17, 1947. By January 22, 1947, winds had caused some defoliation on the north sides of the trees; thereafter, bark samples were taken from both the north and the south sides of the trees. Normal

Fig. 2. Transverse sections (*A, B, C*) of phloem of trunks of sour orange trees grown from seed. *A*, section showing cambium (*c*); uniformly thick ring of functioning phloem (*f*); degenerating phloem (*d*) consisting of only occasional degenerating cells; and some of the nonfunctioning phloem (*nf*) within which are blocks of folded phloem cell walls (*b*) between the rays, some of which have become stretched tangentially and divided (outlined with white ink) to form blocks of parenchymatous tissue in which sclereids (*s*) form. *B*, part of a band of functioning phloem with a band of necrotic sieve tubes (*n*) within the functioning phloem. *C*, a cluster of dead parenchyma cells (*x*) within the functioning phloem (*s.t.* = sieve tube). (*A*, × 45; *B*, × 120; *C*, × 630.)

bands of functioning phloem were present in 32 of 40 of these samples. Degenerating phloem was either present in narrow bands or absent. Unusually wide bands of degenerating phloem 130, 150, and 200μ wide were present in 3 of the other 8 samples. Narrow bands of phloem with necrotic sieve tubes and hypertrophied parenchyma were located tangentially through the functioning phloem of 4 of the samples (fig. 2, *B*). In one sample, necrotic sieve tubes were scattered through the functioning phloem.

TABLE 1

CHARACTERISTICS OF TRUNK PHLOEM OF SWEET AND SOUR ORANGE TREES GROWN FROM SEED, AND OF SWEET ORANGE SCIONS AND SOUR ORANGE ROOTSTOCKS

Phloem samples		Average radial width (μ) of fiber bundles*		Average number of rows of fiber bundles in functioning phloem†		Average width (μ) of band of functioning phloem		Calculated number of fibers in band of functioning phloem 500μ wide	
Source	Total number	Sweet orange	Sour orange	Sweet orange	Sour orange	Sweet orange	Sour orange	Sweet orange	Sour orange
Valencia orange seedlings	20	43	..	2.1	...	761	...	1.4	...
Sour orange seedlings‡	20	..	53	...	0.4	...	488	...	0.4
Valencia orange scion	20	35	..	1.6	...	586	...	1.4	..,
Paraguay sour stock	20	..	52	...	0.6	...	560	...	0.5
Valencia orange scion	18	38	..	1.4	...	479	...	1.5	...
Griffith's sour stock‡	18	..	68	...	0.7	...	592	...	0.6
Navel orange scion	20	41	..	2.7	...	679	...	2.0	...
CES field-12 sour stock‡	20	..	55	...	0.2	...	561	...	0.2
Navel orange sandwich (Valencia orange top)	20	51	..	3.6	...	842	..,	2.1	...
Griffith's sour stock‡	20	..	71	...	0.7	...	695	...	0.5
Average	..	42	60	2.3	0.5	669	579	1.7	0.4

* Measurements made on bundle appearing to be of average thickness in each section.
† Based on sum of rows or partial rows of fibers in functioning phloem of a cross section. If a row of fibers was between the zones of degenerating and functioning phloem, only half of its length was recorded.
‡ All indications were that these belonged to the normal or true sour orange group.

Thirty-nine of 40 samples from five 12-year-old sweet orange seedlings collected on the same dates as the sour orange material exhibited no abnormalities of functioning and degenerating phloem. The functioning phloem of one section had a band of degenerating sieve tubes through it.

In summary it may be stated that in the bark of sour orange seedling trees a wide band of functioning phloem is generally present throughout the winter, together with a narrow band of degenerating phloem which shows no seasonal variation in width. Variations from this pattern in the functioning phloem were observed in 8 of 40 samples, however.

Anatomically, the functioning phloem of the sour orange is much like that of the sweet orange (fig. 1). Bundles of fibers are usually present, as in the sweet orange, but the bundles are wider radially and there are generally fewer of them than in the sweet orange (table 1). Fiber bundles are occasionally isolated rather than in bands (fig. 1).

Phloem of Sour Orange Rootstock of Sweet Orange Trees. In the budded tree, the scion and rootstock of different species may affect the anatomy of each other. For this reason, the phloem of the sour orange stock of sweet orange trees is here considered independently of that of the sour orange seedlings described above.

Rings of functioning phloem about 500μ in width, with only narrow zones of degenerating phloem, were generally present throughout the year in the sour orange rootstocks of these studies. In some samples from the Azusa strain of Valencia orange trees on Paraguay sour stock, however, there was what appeared to be an abnormal degeneration of sieve tubes below the bud union.

Callusing of the sieve plates and degeneration of the older sieve tubes of the ring of functioning phloem immediately below the union were observed in some of the Azusa strain of Valencia orange trees on Paraguay sour orange rootstock. From each tree, pairs of samples were taken, one sample 1½ inches above the bud union and the other 1½ inches below the bud union. Of nine trees sampled, only three were consistently free of such callusing. Fourteen pairs of samples from one of the healthy trees were free of the degeneration on seven different collection dates throughout the year. Six pairs of samples from another healthy tree were free of the degeneration on three collection dates in winter, spring, and summer, as were also two pairs of samples from a third tree on one collection date in July. In contrast to these three trees, six other trees showed callusing of sieve plates and collapse of the older, outer sieve-tube elements on some samplings. Of 23 pairs of samples from these six trees, 10 showed necrosis below the union but not above. Three sample pairs showed necrosis both above and below the union, but the necrosis was more extensive below. In one sample pair there was a wide band of degenerating phloem above the union but not below. (This sample could have been from a different file of cells.) Nine pairs of samples from these trees showed no abnormalities. In roots 18 inches below the union only 1 section out of 23 showed callusing of the outer sieve tubes.

Twenty-three samples from the California normal sour orange rootstocks of six navel orange trees growing in the Citrus Experiment Station orchards showed normal rings of functioning phloem and narrow bands of degenerating phloem, as did also the navel orange scions.

Many samples collected at various times of the year from trees in an orchard near Covina, in which quick decline was spreading, showed no abnormal degeneration of the rootstock or scion phloem except when from trees affected by quick decline.

As was found in the comparison of bark samples from sweet orange and sour orange seedlings, the fiber bundles of the sour orange rootstock are wider than those of the sweet orange scion, and there are generally fewer fiber bundles in the sour orange rootstock (table 1). The band of functioning phloem in the sour orange rootstock tended to be narrower than that in the sweet orange scion.

Normally, bark of the sour orange rootstock under sweet orange trees apparently has a band of functioning phloem about 500μ in width, with only a narrow zone of degenerating phloem on its outer margin. Deviations from

this proposed normal were found, however, in part of the group of Valencia orange trees on Parkinson sour stock. Observations on trees in orchards and greenhouses in this study have shown that this type of injury can become sufficiently extensive and prolonged to provide translocation obstacles and cause trees to go into chronic decline.

DISCUSSION

Materials present in the sieve tubes of the trees as they move across the leaf mosaic may have a toxic or stimulating effect on cells or sensitive elements of the rootstock and vice versa. In commercial practice different varieties of trees or stems and rootstocks are budded together. Some of these combinations may be inherently incompatible or semi-adverse and only witness which cause incompatibility.

In these studies the Arnold strain of Valencia orange on Parkinson sour orange rootstock showed tendencies toward incompatibility in that the older sieve tubes of the bark of functioning phloem were invaded in some samples from below the bud union by quick-decline-affected trees, the younger sieve tubes and the tree to become severe, and a number of secondary symptoms below the initial surface. No decline was observed in trees of the Arnold strain of Valencia orange on sour stock, but a decline in which necrosis of sieve tubes involves not only the oldest, but also the middle-aged and occasionally even young sieve tubes has been observed in some commercial Valencia orchards. This decline, which is tentatively designated "chronic decline," will be discussed in a later paper.

The normal anatomy of the sour orange rootstock under sweet orange is thought to include a uniformity with build of functioning phloem throughout the year. Two types of apparently abnormal phenomena have been observed. Instead of the functioning phloem of the trunk of the sour orange when grown as a seedling or as a rootstock for sweet orange: (1) In some samples from seedling sour orange trees, there were bands of phloem containing necrotic sieve tubes in and contiguous with the functioning phloem. Smaller bands are frequently found as an initial response in the sour orange stock of sweet orange trees affected by quick decline. In the quick-decline trees the altered phloem eventually affects the entire phloem, however, whereas in the seedlings the altered sieve tubes became involved. (2) The other apparently abnormal phenomenon was a development of unusually large numbers of sieve tubes in the outer portion of the functioning phloem. In quick-decline-affected trees necrosis first occurs in the young sieve tubes at the inner portion of the functioning phloem.

SUMMARY

Sieve tube tubes of the functioning phloem of sour orange stock of sweet orange trees budded on sour stock when the tree is affected by the quick-decline virus. The condition of the functioning phloem of the trunk of the healthy sour orange, both as a rootstock and as a seedling tree, is of interest. The functioning phloem of such trunks, especially during the winter months, has been investigated.

Throughout the winter months the trunks of healthy sour orange trees

maintained a band of functioning phloem averaging about 500μ in width. A ring of degenerating phloem external to this was either absent or as much as 100μ wide. Occasionally there were unusually wide bands of degenerating phloem or bands of necrotic sieve tubes within the functioning phloem.

Sour orange rootstocks under sweet orange tops, and usually had a band of functioning phloem averaging 500 or 600μ in width. In one group of trees (Azusa strain of Valencia orange on Paraguay sour orange stock) however there was an abnormal amount of degeneration in the outer part of the functioning phloem.

The trunk phloem of the sour orange, either as seedling trees or as rootstocks for sweet orange trees, had fiber bundles which averaged wider radially than those of the sweet orange but were fewer in number. The width of the band of functioning phloem of the sour orange was, on the average, about 100μ less than that of the sweet orange.

No alterations in anatomy of the trunk phloem of the sour orange were noted as a result of its use as a rootstock except in the Paraguay sour orange rootstock under the Azusa strain of Valencia orange.

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EFFECT OF TRUNK GIRDLING ON PHLOEM OF TRUNK OF SWEET ORANGE TREES ON SOUR ORANGE ROOTSTOCK[1]

HENRY SCHNEIDER[2]

INTRODUCTION

In an anatomical study of quick-decline-affected sweet orange trees on sour orange rootstock, it was noted that degeneration of the older sieve tubes of the functioning phloem immediately above the bud union followed an initial necrosis below the union (Schneider, 1954*a*). This degeneration was less extensive 1 inch above the union than it was immediately above, and little or no degeneration occurred 18 inches above the union until the tree deteriorated.

Degeneration immediately above the union is probably a result of the necrosis of the sieve tubes below the union, and not a result of the action of the virus. This is thought to be true for the following reasons: (1) The phloem of quick-decline-infected sweet orange trees on sweet orange stock is not affected by the disease. (2) The phloem 18 inches up the trunk from the bud union of diseased sweet orange trees on sour stock is not affected in early stages of the disease. (3) Degeneration of sieve tubes and companion cells above the bud union is secondary to the necrosis below the union. (4) Sieve-tube degeneration in peach follows girdling (Schneider, 1945).

Certain varieties of lemon trees on sweet orange, sour orange, grapefruit, mandarin, or Sampson tangelo rootstocks may be affected by lemon tree decline. This decline is brought about by a necrosis of sieve tubes just above the bud union (Schneider, 1948; Calavan *et al.*, 1951). The sieve tubes below the union do not degenerate. In other words, in lemon trees, girdling brought about by sieve-tube necrosis immediately above the bud union does not cause degeneration of sieve tubes below the girdle. This is in contrast to the condition in quick-decline-affected orange trees, in which sieve-tube degeneration above the girdle results from a virus-induced sieve-tube necrosis immediately below the union.

The purpose of the present study was to determine whether or not experimental girdling at the bud union of healthy-appearing sweet orange trees on sour orange rootstock would result in sieve-tube degeneration above the union and not below.

MATERIALS AND METHODS

Two groups of five trees each, growing in the orchard at the Citrus Experiment Station, were used for this study. The trees of one group were the Azusa strain of Valencia orange on Paraguay sour orange rootstock. These

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were vigorous, healthy-appearing 15-year-old trees having dense foliage, but prior to experimental girdling some of them did exhibit a necrosis of the older sieve tubes below the bud union. Degeneration of sieve tubes above the union was sometimes found in trees having necrosis below the union. This was probably a secondary response. Trees of the other group were Washington Navel orange on California standard sour orange rootstocks. These trees were 22 years old and in good condition, although not so vigorous or densely foliated as the trees on Paraguay sour stock.

Every 3 months a ⅜-inch-wide ring of bark was removed at the bud union of one of the trees of each group, until four trees of each group had been girdled. One tree of each group was left as a check. Bark samples for sectioning were taken 1½ inches and 18 inches above and below the bud union on the girdling date and 2 weeks, 6 weeks, 3 months, 9 months, and 1 year after girdling. Starch tests were made on finger-sized roots by cutting across them with pruning shears and applying a solution of iodine in potassium iodide, a method described by Fawcett (1945) for testing starch in roots. Color intensities were then recorded. The condition of the tops of the trees was recorded at the time of sampling.

OBSERVATIONS

Anatomic Alterations Induced by Ringing. In healthy trees the bands of developing and degenerating phloem are usually narrow in radial width. In ringed trees a portion of the outer functioning phloem degenerates. This is referred to here as *induced degenerating phloem.*

Results presented in tables 1 and 2 were obtained by dividing the radial width of functioning phloem, expressed in microns, by the combined widths of functioning phloem and induced degenerating phloem, and multiplying the quotient by 100. This gives the percentage of phloem that remains functioning, based on the amount that would normally be present. Since the induced degenerating phloem becomes part of the nonfunctioning phloem, the values are an inaccurate measure of phloem degeneration after trees have been ringed for considerable time. The percentage of functioning phloem based on what would be present if degeneration had not been induced is preferable, however, to measurements of phloem actually functioning, because of the normal variation in amount of functioning phloem.

Unfortunately, even before the ringing, sieve-tube degeneration from an unknown cause was evident in the phloem of some of the samples from below the bud union of three of the five Valencia orange trees on Paraguay sour stock, as indicated in table 2. It should also be pointed out that occasionally there may be excessively wide bands of degenerating phloem in apparently healthy orange trees (Schneider, 1954*b*).

From the data presented in tables 1 and 2, it is obvious that girdling a tree by removing a ring of bark at the bud union induced degeneration of the older sieve tubes in other parts of the trunk. The degeneration first occurred in about equal amounts 1½ inches above and below the girdle. The more distant points (18 inches above and below the bud union) were usually not affected until later.

The season of the year in which the girdling was done had a marked effect

on the time required for the degeneration to begin to appear. When trees were girdled in October, degeneration was first observed on the collection date 9 months later; when they were girdled in January, degeneration was observed 3 months later. Six weeks were required for response when the trees were girdled in April, and only 2 weeks when girdled in July. Apparently, response to girdling does not occur between October and April.

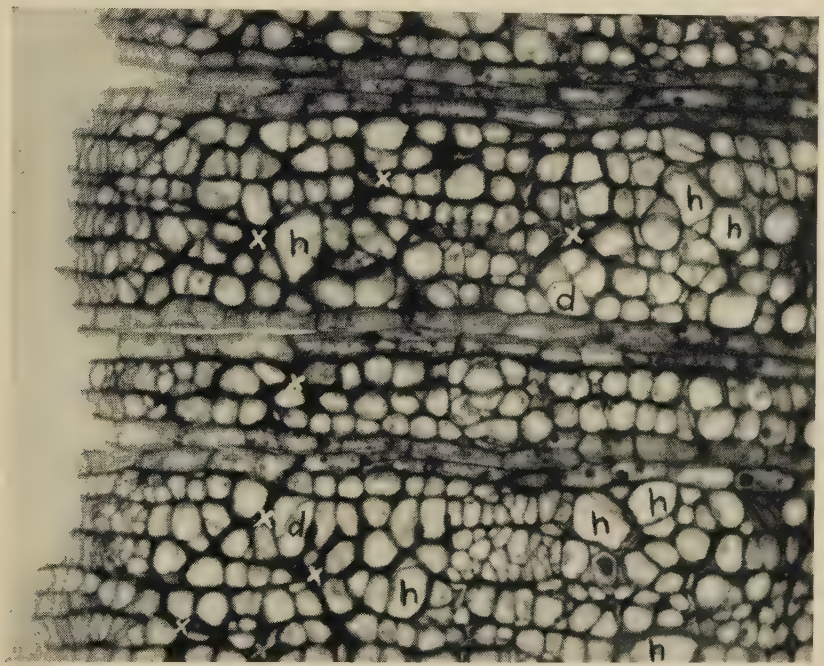

Fig. 1. Cross section of a root from a girdled tree, showing crushed sieve tubes (*x*) and hypertrophied (*h*) and divided (*d*) parenchyma cells, which sometimes occur. The cambium is at the left. (× 260.)

Induced degeneration of sieve tubes proceeded in a normal manner: Callus formed on sieve plates, the sieve-tube elements collapsed, and then the sieve-tube contents and callus were removed. In some cases hypertrophy of parenchyma cells accompanied sieve-tube necrosis. Hypertrophy was observed to be extensive in 12 roots (fig. 1) and occasional in 6 others. About half of the roots having sieve-tube degeneration showed some hypertrophy. In 11 of the trunk samples taken 1½ inches below the bud union, there was a moderate amount of hypertrophy, and in 35 samples there was a slight amount. At this point of sampling there was hypertrophy of parenchyma cells in about two thirds of the samples which had degenerating sieve tubes. At 1½ inches above the bud union, 11 of 68 trunk samples having degenerating sieve tubes exhibited occasional hypertrophy of the parenchyma; and at 18 inches above, 5 of 50 samples having degenerating sieve tubes also showed occasional hypertrophy.

In summary, hypertrophy of parenchyma cells accompanying sieve-tube

TABLE 1

PERCENTAGE OF PHLOEM WHICH REMAINED FUNCTIONAL IN TRUNKS OF WASHINGTON NAVEL ORANGE TREES ON CALIFORNIA STANDARD SOUR ORANGE ROOTSTOCKS AT INTERVALS AFTER GIRDLING (BY RINGING) AT BUD UNION

Bark sampling		Percentage of phloem functioning, as indicated by bark samples (A and B) from opposite sides of tree													
Direction from bud union	Distance from bud union (inches)	On date of girdling		After 2 weeks		After 6 weeks		After 3 months		After 6 months		After 9 months		After 1 year	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B
Tree 13-12, girdled January 24, 1946															
		1-24-46		2-8-46		3-8-46		4-23-46		7-22-46		10-21-46		1-20-47	
Above	18	100	100	100	100	100	100	100	100	100	100	100	100	70	90
	1½	100	100	100	100	100	100	80	60	30	30	40	20	30	50
Below	1½	100	100	100	100	100	100	70	70	30	50	10	20	10	0
	18	100	100	100	100	100	100	100	100	100	70	20	0	70	0
Tree 14-13, girdled April 23, 1946															
		4-23-46		5-7-46		6-6-46		7-22-46		10-21-46		1-20-47		4-22-47	
Above	18	100	100	...	...	70	100	90	70	100	75	60	100	70	100
	1½	100	100	100	100	30	80	20	20	20	50	30	30	30	40
Below	1½	100	100	100	100	60	60	60	70	0	50	0	20	30	30
	18	100	100	...	...	100	100	100	100	100	0	0	100	70	70

Tree 13-13, girdled July 22, 1946

		7-22-46		8-5-46		8-30-46		10-21-46		1-20-47		4-22-47		7-22-47	
Above	18	100	100	80	80	70	70	50	80	40	40	50	60	60	30
	1½	100	100	30	50	40	40	30	50	20	40	30	40	10	10
Below	1½	100	100	30	60	40	40	40	40	50	40	50	50	40	40
	18	100	100	100	100	0	100	30	100	100	100	100	30	30	30

Tree 14-11, girdled October 21, 1946

		10-21-46		11-5-46		12 4-46		1-20-47		4 22 47		7-22-47		10-24 47	
Above	18	100	100	...	...	100	100	66	100	50	80	40	70	60	70
	1½	100	100	100	100	100	100	100	100	100	100	20	40	30	20
Below	1½	100	100	100	100	100	100	100	100	100	100	70	30	10	30
	18	100	100	...	...	100	100	100	100	100	100	100	70	90	70

Check trees (not girdled)

		2-8-46		3-8-46		6-6-46		8-30-46		12-4-46		1-20-47		4-22-47	
Above	18	100	100	100	...	100	100	100	100	100	100	100	100	100	100
	1½	100	100	100	...	100	100	100	100	100	100	100	100	100	100
Below	1½	100	100	100	...	100	100	100	100	70	100	100	100	100	100
	18	100	100	100	...	100	100	100	100	100	100	100	100	100	100

TABLE 2

PERCENTAGE OF PHLOEM WHICH REMAINED FUNCTIONAL IN TRUNKS OF VALENCIA ORANGE TREES ON PARAGUAY SOUR ORANGE ROOTSTOCK AT INTERVALS AFTER GIRDLING (BY RINGING) AT BUD UNION

Bark sampling		Percentage of phloem functioning, as indicated by bark samples (A and B) from opposite sides of tree													
Direction from bud union	Distance from bud union (inches)	On date of girdling		After 2 weeks		After 6 weeks		After 3 months		After 6 months		After 9 months		After 1 year	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B
Tree 17-2, girdled January 24, 1946															
		1-24-46		2 8-46		3-8-46		4-23 46		7-22-46		10 21 46		1-20 47	
Above	18	100	100	100	100	100	100	100	100	80	70	100	100	80	40
	1½	100	50*	100	100	100	50*	50	100	50	20	20	50	30	50
Below	1½	50*	30*	50*	50*	100	20*	10	70	70	60	80	60	50	100
	18	100	100	100	100	100	100	100	100	70	90	70	50	90	70
Tree 17-5, girdled April 23, 1946															
		4-23-46		5 7-46		6-6-46		7 22 46		10 21-46		1 20-47		4-22-47	
Above	18	80	90	...	...	70	50	100	100	60	100	60	100	60	100
	1½	60*	100	80*	80*	60	50	20	30	30	20	30	30	20	40
Below	1½	20*	60*	30*	90*	40	70	60	80	40	60	50	100	50	50
	18	100	40	...	...	100	0	100	100	100	70	70	100	100	100

Tree 17-4, girdled July 22, 1946

		7-22-46		8-5-46		8-30-46		10-21-46		1-20-47		4-22-47		7-22-47	
Above	18	100	100	90	40	80	60	60	60	50	80	100	100	30	50
	1½	100	100	40	50	30	70	40	60	40	70	30	60	40	30
Below	1½	100	100	60	80	60	10	80	10	50	10	50	70	20	30
	18	100	100	100	100	70	60	100	100	80	100	100	100	100	30

Tree 17-3, girdled October 22, 1946

		10-21-46		11-5-46		12-4-46		1-20-47		4-22-47		7-22-47		10-24-47	
Above	18	100	100	...	...	100	100	100	100	100	100	50	60	30	40
	1½	100	100	100	100	100	100	100	100	100	100	20	30	10	20
Below	1½	60*	100	40*	100	100	100	100	100	100	100	70	30	40	40
	18	100	100	...	...	100	100	100	100	100	100	100	100	30	30

Check trees (not girdled)

		2-8-46		3-8-46		6-6-46		8-30-46		12-4-46		1-20-47		4-22-47	
Above	18	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	1½	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Below	1½	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	18	100	100	100	100	100	100	100	100	100	100	100	100	100	100

* Sections probably showing degeneration from some cause other than girdling by ringing.

degeneration in the phloem was most severe 18 inches below the bud union (in roots), less severe 1½ inches below the union, and only slight and infrequent at points above the union.

Time Required for Starch Depletion in Roots and for Appearance of Top Symptoms. Starch tests were made on roots from opposite sides of the tree. Finger-sized roots were cut with pruning shears in such a way as to crush the cells slightly in the process; iodine in potassium iodide was then applied, and color was noted.

The period required for starch depletion of the roots ranged from 6 weeks to 6 months and depended on the date of tree ringing. The least time (about 6 weeks) was required when ringing was done on July 22; the longest time (6 months), when ringing was done on October 21. Fawcett (1946) reported that roots of trees ringed on February 6, 1945, were almost depleted of starch 5 months later.

The time required for deterioration of the tree and the appearance of top symptoms—namely, yellowing and loss of leaves—ranged from 3 months to more than 6 months. Trees ringed in the spring and summer required the least time (3 to 6 months); those ringed in October required more than 6 months.

DISCUSSION

Presumably, in an evergreen tree there is a continuous downward movement of carbohydrates through the trunk phloem to the roots. When a tree is girdled, the sieve tubes above the girdle cease to have any place to transport materials, and carbohydrates therefore accumulate above the girdle. Sieve tubes below the girdle still have some function to perform, however. Although the amount of available carbohydrates is drastically lowered, these sieve tubes can still translocate the carbohydrates stored as starch in the woody part of the trunk to the roots.

Sieve-tube degeneration induced above girdling brought about by the removal of a ring of bark at the bud union of a healthy orange tree seems to be identical with that induced above girdling brought about naturally by the necrosis of sieve tubes immediately below the bud union of a quick-decline tree.

What happens to sieve tubes below a girdle in orange trees girdled by removal of a ring of bark, and in lemon trees girdled by necrosis of sieve tubes, is strikingly different. Degeneration occurred below the artificial girdle in orange trees, but not below the naturally occurring girdle in lemon trees. Effects of girdling by ringing differ in at least two respects from those of naturally occurring girdling caused by sieve-tube necrosis: (1) There is a wound involved in ringed trees, and there may be wound responses even 1½ inches below the union. (2) A few of the sieve tubes and all of the parenchyma cells remain intact in lemon trees naturally girdled by sieve-tube necrosis. These cells and sieve tubes may serve to let sufficient hormones and vitamins pass the girdle, even though the sieve-tube necrosis critically limits carbohydrate translocation. In trees artificially girdled by ringing, translocation of materials in the bark is completely stopped.

SUMMARY

The effect of girdling, by removing a ring of bark at the bud union, upon the condition of the phloem of the remainder of the trunk and roots of sweet orange trees on sour orange rootstock has been studied.

Artificial girdling of orange tree trunks caused degeneration of sieve tubes, the older sieve tubes being affected first and the younger ones later.

Degeneration usually occurred sooner and more extensively 1½ inches above and below the union than it did 18 inches above and below the union. Severe degeneration of sieve tubes and hypertrophy of parenchyma occurred in some roots in advanced cases, however.

The time required for sieve-tube degeneration to begin depended on the season of year in which the girdling was done. Two weeks were required for a response when trees were girdled in July, and more than 6 months when girdled in October.

The reserve starch in finger-sized roots of one tree ringed on July 22 was depleted after 6 weeks, but 6 months were required for trees ringed on October 21 to utilize their reserve starch.

Sieve-tube degeneration 1½ and 18 inches above the artificial girdle in healthy orange trees was similar in time of occurrence and mode of degeneration to that found in orange trees affected by quick decline.

Degeneration 1½ inches below the artificial girdle in orange trees was contrary to a condition recently found in lemon trees on various rootstocks, in which the sieve tubes of the scion become necrotic but those of the stock immediately below the union remain functional.

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